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Michael O. Leavitt, Administrator
U.S. Environmental Protection Agency
Ariel Rios Bldg. (1101A)
1200 Pennsylvania Ave. NW
Washington, DC 20460

Comments on the HPV test plan for nitric acid 2-ethylhexyl ester (2-EHN)

Dear Administrator Leavitt:

The following comments are on the test plan for 2-EHN (CAS no. 27247-96-7), prepared by the American Chemistry Council (ACC). These comments are submitted on behalf of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, the Humane Society of the USA, the Doris Day Animal League, and Earth Island Institute. These health, animal-protection and environmental organizations have a combined membership of more than ten million Americans.

The ACC states that it intends to carry out a "reproduction/developmental toxicity study" on 2-EHN. On the assumption that this refers to OECD test no. 421 or 422, this study will involve the killing at least 675 mammals.

The ACC should have provided more information on the use of, and human exposure to, this diesel additive. Taking into consideration the probable low concentration of 2-EHN in diesel, together with its low general toxicity (the LD_{50} for oral administration to rats is greater than 10 mL/kg and the 28-day no-observed-effect level is 20 mg/kg/day), it is possible that in real-world terms it is highly unlikely that 2-EHN will have any toxicity other than that of diesel fuel itself.

In addition, one of the four repeat-dose studies to which the ACC has referred, the 21-day dermal study in rabbits, appears to have involved the collection of reproductive toxicity data, as the effects of 2-EHN on the ovaries and testes were evaluated (robust summaries, p. 17). However, the report of this study is "unpublished confidential business information," and we therefore do not know whether the findings were directly relevant to reproductive toxicity endpoints. We also note that toxicity data are not considered CBI under normal circumstances.

As an alternative to the *in vivo* reproductive and developmental toxicity test, we recommend that the ACC perform the rodent embryonic stem cell test (EST). This *in vitro* embryotoxicity method has been validated by the European Centre for the Validation of Alternative Methods (ECVAM), and the Centre's Scientific Advisory Committee has concluded that the test is ready to be considered for regulatory purposes (Genschow 2002). We have repeatedly provided validation and SOP references, and we have suggested that, in the HPV program, which is supposed to be a *screening-level* program, a positive EST results should warrant the substance's treatment as a developmental toxicant, so that no further testing should be carried out.

If the ACC insists on carrying out OECD test 421, we urge it to consider performing the EST in parallel. Several companies have agreed to do so in order to help build a database for industrial chemicals, for eventual validation of the EST in the USA. We are still awaiting a response from the ACC to our February 25 letter on this matter.

Finally, the ACC states that it intends to carry out a chromosomal aberration study but does not provide details. Per EPA guidance, this test should be conducted *in vitro*. We also urge the ACC to use human lymphocytes or mammalian cells obtained from established cultures, so as to avoid killing additional animals.

Please feel free to contact me at 757-622-7382, ext. 8001, or via e-mail at JessicaS@peta.org.

Sincerely,

Jessica Sandler
Federal Agency Liaison

References

Genschow, E., "The ECVAM international validation study on *in vitro* embryotoxicity tests: Results of the definitive phase and evaluation of prediction models", *Alternatives to Laboratory Animals* 30: 151-176, 2002.